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(54) Title: GLUCOSIDASES, NUCLEIC ACIDS ENCODING THEM AND METHODS FOR MAKING AND USING THEM

(57) Abstract: The invention is directed to polypeptides having a glucosidase activity, including an alpha-glucosidase activity, polynucleotides encoding the polypeptides, and methods for making and using these polynucleotides and polypeptides. In one aspect, the polypeptides of the invention are used as alpha-glucosidases to catalyze the hydrolysis of starch into sugars, e.g., to convert liquefied starch to glucose. In one aspect, the polypeptides of the invention can catalyze the hydrolysis of both alpha-(1,4) and alpha-(1,6) glucose linkages. In one aspect, the polypeptides of the invention can catalyze the hydrolysis of both malto-oligosaccharides and liquefied starch.

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A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12P 21/06; C12N 9/00, 9/24, 1/20, 15/00; C11D 3/50; C07K 16/00; C07H 21/04
US CL : 435/69.1, 183, 200, 252.3, 287.2, 320.1; 510/114, 392; 530/387.1, 830; 536/23.2; 712/1, 90
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/69.1, 183, 200, 252.3, 287.2, 320.1; 510/114, 392; 530/387.1, 830; 536/23.2; 712/1, 90

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 6,355,467 (KELLY et al.)12 March 2002 (12.03.2002) see entire document.	52-71, 74-82, 99, 103-128, 207-208 --- 72-73, 83-98, 100-102, 154-191, 209-218
X --- Y	ROLFSMEIER et al. Purification and characterization of a maltase from extremely thermophilic crenarchaeote S.solfataricus. J. Bacteriol., 1995, Vol. 177, No.2, pages 482-485.	52-71, 74-82, 99, 103-128, 207-208 --- 72-73, 83-98, 100-102, 154-191, 209-218
X --- Y	LEGIN E et al. production of thermostable amylolytic enzymes by T.hydrothermalis. Biotechnol. Lett., April 1998, Vol.20, No.4, pages 363-367. see entire document.	52-71, 74-82, 99, 103-106, 107-128, 207-208 --- 72-73, 83-98, 100-102, 154-191, 209-218

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	KLINGEBERG M et al. Production of novel pullulanases at high concentrations by two newly isolated thermophilic clostridia. FEMS Microbiol. Lett., Mqy 1990, Vol. 57, No.1-2, pages 145-52.	52-71, 74-82, 99, 103-106, 107-128, 207-208 --- 72-73, 83-98, 100-102, 154-191, 209-218
X --- Y	Database GenBank, US National Library of Medicine, (Bethesda, MD, USA) Accession No.AE011901, deSILVA ACR et al., 29 May 2002.	1-2, 5-15, 23-39, 47-48 --- 49-51, 129-153, 193-206

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-218, SEQ ID NO:1 and 2 only

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

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BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-218, drawn to an isolated polynucleotide with SEQ ID NO:1 encoding a polypeptide with SEQ ID NO:2, vectors, host cells, antisense DNA, RNA, method of using the polynucleotides and polypeptide, a transgenic animal, a transgenic plant/seed, a computer system and media comprising said polynucleotide or polypeptide, a method of making a small using the polypeptide, a method for whole cell engineering using the polynucleotide /polypeptide, an antibody that interacts with the above polypeptide and methods of generating the same, an isolated signal sequence comprising 1-32 amino acids of the polypeptide.

Group II, claims 1-218, drawn to an isolated polynucleotide with SEQ ID NO:3 encoding a polypeptide with SEQ ID NO:4, vectors, host cells, antisense DNA, RNA, method of using the polynucleotides and polypeptide, a transgenic animal, a transgenic plant/seed, a computer system and media comprising said polynucleotide or polypeptide, a method of making a small using the polypeptide, a method for whole cell engineering using the polynucleotide /polypeptide, an antibody that interacts with the above polypeptide and methods of generating the same, an isolated signal sequence comprising 1-32 amino acids of the polypeptide.

Group III, claims 1-218, drawn to an isolated polynucleotide with SEQ ID NO:5 encoding a polypeptide with SEQ ID NO:6, vectors, host cells, antisense DNA, RNA, method of using the polynucleotides and polypeptide, a transgenic animal, a transgenic plant/seed, a computer system and media comprising said polynucleotide or polypeptide, a method of making a small using the polypeptide, a method for whole cell engineering using the polynucleotide /polypeptide, an antibody that interacts with the above polypeptide and methods of generating the same, an isolated signal sequence comprising 1-32 amino acids of the polypeptide.

Group IV, claims 1-218, drawn to an isolated polynucleotide with SEQ ID NO:7 encoding a polypeptide with SEQ ID NO:8, vectors, host cells, antisense DNA, RNA, method of using the polynucleotides and polypeptide, a transgenic animal, a transgenic plant/seed, a computer system and media comprising said polynucleotide or polypeptide, a method of making a small using the polypeptide, a method for whole cell engineering using the polynucleotide /polypeptide, an antibody that interacts with the above polypeptide and methods of generating the same, an isolated signal sequence comprising 1-32 amino acids of the polypeptide.

Group V, claims 1-218, drawn to an isolated polynucleotide with SEQ ID NO:9 encoding a polypeptide with SEQ ID NO:10, vectors, host cells, antisense DNA, RNA, method of using the polynucleotides and polypeptide, a transgenic animal, a transgenic plant/seed, a computer system and media comprising said polynucleotide or polypeptide, a method of making a small using the polypeptide, a method for whole cell engineering using the polynucleotide /polypeptide, an antibody that interacts with the above polypeptide and methods of generating the same, an isolated signal sequence comprising 1-32 amino acids of the polypeptide.

Group VI, claims 1-218, drawn to an isolated polynucleotide with SEQ ID NO:11 encoding a polypeptide with SEQ ID NO:12, vectors, host cells, antisense DNA, RNA, method of using the polynucleotides and polypeptide, a transgenic animal, a transgenic plant/seed, a computer system and media comprising said polynucleotide or polypeptide, a method of making a small using the polypeptide, a method for whole cell engineering using the polynucleotide /polypeptide, an antibody that interacts with the above polypeptide and methods of generating the same, an isolated signal sequence comprising 1-32 amino acids of the polypeptide.

Group VII, claims 1-218, drawn to an isolated polynucleotide with SEQ ID NO:13 encoding a polypeptide with SEQ ID NO:14, vectors, host cells, antisense DNA, RNA, method of using the polynucleotides and polypeptide, a transgenic animal, a transgenic plant/seed, a computer system and media comprising said polynucleotide or polypeptide, a method of making a small using the polypeptide, a method for whole cell engineering using the polynucleotide /polypeptide, an antibody that interacts with the above polypeptide and methods of generating the same, an isolated signal sequence comprising 1-32 amino acids of the polypeptide.

Group VIII, claims 1-218, drawn to an isolated polynucleotide with SEQ ID NO:15 encoding a polypeptide with SEQ ID NO:16, vectors, host cells, antisense DNA, RNA, method of using the polynucleotides and polypeptide, a transgenic animal, a transgenic plant/seed, a computer system and media comprising said polynucleotide or polypeptide, a method of making a small using the polypeptide, a method for whole cell engineering using the polynucleotide /polypeptide, an antibody that interacts with the above polypeptide and methods of generating the same, an isolated signal sequence comprising 1-32 amino acids of the polypeptide.

Group IX, claims 1-218, drawn to an isolated polynucleotide with SEQ ID NO:17 encoding a polypeptide with SEQ ID NO:18, vectors, host cells, antisense DNA, RNA, method of using the polynucleotides and polypeptide, a transgenic animal, a transgenic plant/seed, a computer system and media comprising said polynucleotide or polypeptide, a method of making a small using the polypeptide, a method for whole cell engineering using the polynucleotide /polypeptide, an antibody that interacts with the above polypeptide and methods of generating the same, an isolated signal sequence comprising 1-32 amino acids of the polypeptide.

Group X, claims 1-218, drawn to an isolated polynucleotide with SEQ ID NO:19 encoding a polypeptide with SEQ ID NO:20, vectors, host cells, antisense DNA, RNA, method of using the polynucleotides and polypeptide, a transgenic animal, a transgenic plant/seed, a computer system and media comprising said polynucleotide or polypeptide, a method of making a small using the polypeptide, a method for whole cell engineering using the polynucleotide /polypeptide, an antibody that interacts with the above polypeptide and methods of generating the same, an isolated signal sequence comprising 1-32 amino acids of the polypeptide.

Group XI, claims 1-218, drawn to an isolated polynucleotide with SEQ ID NO:21 encoding a polypeptide with SEQ ID NO:22, vectors, host cells, antisense DNA, RNA, method of using the polynucleotides and polypeptide, a transgenic animal, a transgenic plant/seed, a computer system and media comprising said polynucleotide or polypeptide, a method of making a small using the polypeptide, a method for whole cell engineering using the polynucleotide /polypeptide, an antibody that interacts with the above polypeptide and methods of generating the same, an isolated signal sequence comprising 1-32 amino acids of the polypeptide.

Group XII, claims 1-218, drawn to an isolated polynucleotide with SEQ ID NO:23 encoding a polypeptide with SEQ ID NO:24, vectors, host cells, antisense DNA, RNA, method of using the polynucleotides and polypeptide, a transgenic animal, a transgenic plant/seed, a computer system and media comprising said polynucleotide or polypeptide, a method of making a small using the polypeptide, a method for whole cell engineering using the polynucleotide /polypeptide, an antibody that interacts with the above polypeptide and methods of generating the same, an isolated signal sequence comprising 1-32 amino acids of the polypeptide.

The inventions listed as Groups I-XII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
Group I is drawn to polynucleotide encoding a polypeptide having the special technical feature of SEQ ID NO:1 and 2 which groups II-XII do not have.
Group II is drawn to polynucleotide encoding a polypeptide having the special technical feature of SEQ ID NO:3 and 4 which groups I and III-XII do not have.
Group III is drawn to polynucleotide encoding a polypeptide having the special technical feature of SEQ ID NO:5 and 6 which groups I-II and IV-XII do not have.
Group IV is drawn to polynucleotide encoding a polypeptide having the special technical feature of SEQ ID NO: 7 and 8 which groups I-III and V-XII do not have.
Group V is drawn to polynucleotide encoding a polypeptide having the special technical feature of SEQ ID NO:9 and 10 which groups I-IV and VI-XII do not have.
Group VI is drawn to polynucleotide encoding a polypeptide having the special technical feature of SEQ ID NO:11 and 12 which groups I-V and VII-XII do not have.
Group VII is drawn to polynucleotide encoding a polypeptide having the special technical feature of SEQ ID NO:13 and 14 which groups I-VI and VIII-XII do not have.
Group VIII is drawn to polynucleotide encoding a polypeptide having the special technical feature of SEQ ID NO:15 and 16 which groups I-VII and IX-XII do not have.
Group IX is drawn to polynucleotide encoding a polypeptide having the special technical feature of SEQ ID NO:17 and 18 which groups I-VIII and X-XII do not have.
Group X is drawn to polynucleotide encoding a polypeptide having the special technical feature of SEQ ID NO:19 and 20 which groups I-IX and XI-XII do not have.

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Group XI is drawn to polynucleotide encoding a polypeptide having the special technical feature of SEQ ID NO:21 and 22 which groups I-X and XII do not have.
Group XII is drawn to polynucleotide encoding a polypeptide having the special technical feature of SEQ ID NO:23 and 24 which groups I-XI do not have.

Furthermore, The ISA considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first-recited product, a
polynucleotide encoding a polypeptide, a vector, a host cell, a method for producing and several methods of using polypeptide.
Furthermore the ISA considers that any feature which the subsequently recited products and methods share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.

Continuation of B. FIELDS SEARCHED Item 3:
CAPLUS,BIOSIS,SCISEARCH,BIOTECHABS,BIOTECHDS,DGENE,PASCAL,CABA,LIFESCI,USPTO-WEST,BIOTECHNO,GENBANK, AGRICOLA,EMBASE,MEDLINE,ESBIOBASE,FSTA,